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INTRODUCTION

During the last few years certain species of *Crepis* have been used rather widely in cytological investigations and to some extent in genetical investigations as well. This has been due mainly to the low number and the well marked individuality of the chromosomes in the somatic nuclear plates. *Crepis* in these respects is a unique genus, especially because the parentage of spontaneous hybrids in several cases can be determined by the morphology of the chromosomes, as has been shown by M. Navashin (1926, 1927*a*, *b*).

The published investigations on the chromosome numbers of *Crepis* have been mainly the work of Rosenberg (1909, 1918, 1920), of the Berkeley group, summarized by Mann (1925) and by Babcock and Lesley (1926), and of M. Navashin (1925, 1926, 1927). The last mentioned author has specialized on the morphology of the chromosomes. Others who have contributed are Juel (1905), Tahara (1910), Miss Digby (1914), and Marchal (1920).

As the chromosomes of *Crepis* are especially satisfactory for investigation during somatic divisions, most attention hitherto has been paid to this phase. Rosenberg, however, investigated the divisions during the formation of pollen mother cells; Navashin (1927*b*) made some observations on the reduction division of *C. capillaris* × *C. aspera;* while Lesley and Hollingshead have prosecuted a number of (unpublished) investigations on the reduction division of certain interspecific hybrids of *Crepis.* But no detailed study of the course of the reduction division has been previously reported.

The present study comprises the two species, *Crepis aspera* L. and *C. bursifolia* L., together with the three hybrids: *Crepis aspera* ×

C. bursifolia, *C. taraxacifolia* Thuill. × *C. tectorum* L., and *C. aspera* × *C. aculeata* (DC.) Boiss. The five species used for the crossings have $n = 4$ chromosomes. The chromosomes in the four species are very similar in size during meiosis, except in *C. bursifolia*, where one chromosome is conspicuously shorter than the others and different from them.

The material for the present investigations was procured by the senior author, who made the taxonomic studies and wrote section 5, while the junior author made the cytological investigations and wrote the remainder of the paper. We acknowledge with gratitude our indebtedness to Mr. C. W. Haney, who made the crossings and provided observations on the fertility of the hybrids, and to Miss L. Hollingshead, who prepared the fixations of *C. taraxacifolia* × *tectorum* and *C. aspera* × *aculeata*. Miss Hollingshead also made pollen counts of the hybrids. The cytological investigations were carried out during the stay of the junior author at the Division of Genetics, University of California, as a Research Fellow of the International Education Board.

METHODS

The material of *Crepis taraxacifolia* × *tectorum* and *C. aspera* × *aculeata* was fixed in Carnoy's fluid. This material could not be used for paraffin sections and subsequent staining with Heidenhain's haematoxylin or with iodine-gentian violet because the stain disappeared from the chromosomes as soon as from the plasm, making differentiation impossible. For these hybrids Belling's iron-acetocarmine method was successfully applied by Hollingshead to *Crepis* material fixed in Carnoy in order to have reserve material for later examinations and also in order to overcome the difficulty involved in finding time enough to do all the cytological investigations during the period in which reduction division takes place.

The acetic acid of the acetocarmine causes the pollen mother cells and the chromosomes to expand, thus overcoming the contraction and collapsing commonly caused in species hybrids by the Carnoy fixation; and in many cases the acetocarmine gives a clear differentiation between chromosomes and plasm, especially when Belling's monochromatic green light is applied. For the large chromosomes of *Crepis* the differentiation gained through the acetocarmine method is sufficiently distinct to determine pairs and univalents of chromosomes, and in determining the tetrad stages it is much more distinct and

reliable than the paraffin-section method. The disadvantages of the smear method are that it wastes much material and therefore cannot be applied to plants with so few pollen mother cells in the anthers as hybrids often have; also the acetocarmine smears do not give permanent slides, thus making it difficult later on to check up the results previously obtained. The advantage of the iron-acetocarmine method is the quickness with which it can be handled. In many cases, however, the time used for fixing and preparing the sectioned slides is so small compared with the time required for examination of the slides that it does not pay to save a little time in preparing the slides, only to waste it during the examination. Sectioned material is unquestionably much more convenient for examination than smears, where disorder reigns, where seriation of stages is destroyed, and where irregularities of tapetum cells and pollen mother cells, often found in hybrids, escape observation because the general disorder makes it impossible to determine to which type of tissue a certain cell belongs.

In the hybrid *Crepis aspera* × *bursifolia* the following combinations of fixation and staining have been compared:

Fixations	Stainings
Carnoy (absolute alcohol-chloroform-glacial acetic-acid 24 hours)	Heidenhain's iron-haematoxylin, iodine-gentian violet (sections); iron-acetocarmine (smears)
Carnoy-Navashin (Carnoy 5 minutes, followed by Navashin's formalin-chromic-acetic acid 24 hours)	iodine-gentian violet-orange (sections); iron-acetocarmine (smears)

As a fixative, the combination of Carnoy's + Navashin's fluid is much superior to Carnoy alone, since the latter causes too great contraction and destroys too much of the finer structure. Navashin's fluid alone has too little penetrating power and results too often in insufficient fixation, except when it is applied to very small and delicate objects such as root tips. In *Viola* hybrids, furthermore, the junior author has been able to compare Navashin's fixative with the combination of Carnoy's and Navashin's fluids and the latter has proved, in a number of cases and for this material, to be much better than Carnoy alone. It preserves the fine structural details and the objects are thoroughly fixed. In *Crepis* this fixative together with the iodine-gentian violet staining gave clear indication of the inner structure of the chromosomes.

The buds are put in the Carnoy fixative and, as for *Crepis,* the tips of the involucral scales are removed in order to facilitate penetration of the fixative between the florets. The buds remain in the first fixative from three to ten minutes, when they are changed into Navashin's formalin-chromic-acetic acid. Carnoy's fluid seems to prepare the way through the tissue for the second fixative, but in the short time of application it does not dehydrate or collapse the tissue. Kihara (1924) describes a combination Carnoy-Flemming's fluid which is based on the same principle as the combination here described.

As for the stains, Heidenhain's is too little transparent for *Crepis* chromosomes which usually must be counted in side view. Both iodine-gentian violet and iron-acetocarmine (in smears) give good transparent stains, but of these two the gentian violet gives the most contrast and the clearest, most unquestionable counts. Iron-acetocarmine applied on Carnoy-fixed material does not seem to be inferior to the fresh fixed and stained smears. The Carnoy-Navashin fixative is not so adequate for iron-acetocarmine smears as the Carnoy fluid, because the material is not brittle enough to be pulled out in very small, thin pieces. Perhaps the application of absolute alcohol for some time would give the Carnoy-Navashin fixed material the consistency that is necessary for making good smears.

The best way of applying the gentian violet for *Crepis* was to omit the iodine treatment before the staining, going directly from 70 per cent alcohol to 1 per cent gentian violet in water, applied for 5 minutes. After rinsing in water the slides were put in a solution of 1 gram iodine and 1 gram iodide of postassium in 100 cc. of 70 per cent alcohol. Here they stayed for 30 to 45 seconds and thereafter they were rushed through 70 per cent, 95 per cent, and absolute alcohol to pure clove oil with some orange G. Here the differentiation took place and after washing in xylol with a few drops of absolute alcohol in it they were put in pure xylol and afterwards mounted in balsam. The differentiation is often so perfect that the plasm is totally unstained if no orange G has been applied. In sectioned material it is necessary that one be able to see the limits up and down of the section in order to be sure that the pollen mother cell has not been cut. The orange G stains the plasm sufficiently to make its structure visible and still it gives so good a contrast to the gentian-violet stained and transparent chromosomes that these are very clearly differentiated from the plasm.

In some cases, as for example in many species of *Viola*, it is impossible to stain Carnoy-fixed material with gentian violet. Also it is sometimes better to apply the iodine *before* the staining, when the objects have been fixed in Carnoy. The iodine seems to have the effect of binding the stain to the tissue. But in many cases it binds the stain too much and it is impossible to get it out of the plasm again. The use of iodine and gentian violet can be varied in many ways. The method is also very dependent upon the fixative used. Neither in *Viola* nor in *Crepis* was any ease found where iodine-gentian violet failed on material fixed in Navashin's or in Carnoy-Navashin's fluid.

In order to try the effeet of acetic acid, some slides were treated for half an hour with 45 per cent acetic acid. The pollen mother cells swelled somewhat. The acetic aeid did not destroy the gentian violet applied for staining but the differentiation was not so good as in the slides not treated with aeetic aeid. Some sectioned slides were stained with iron-acetocarmine but the effect was much inferior to that of iron-acetocarmine applied on smears. There was too little differentiation between ehromosomes and plasm.

The ideal method for *Crepis* seems to be to have root tips fixed in Navashin's fluid and stained with Heidenhain's haemotoxylin (Navashin 1925, 1926), to have younger buds fixed in Carnoy-Navashin and stained with iodine-gentian violet-orange G for examination of the prophases, the heterotypic, and the homotypic divisions, and to have older buds fixed in Carnoy for examination of tetrad stages in iron-acctoearmine.

MEIOSIS IN CREPIS ASPERA L. AND C. BURSIFOLIA L.

The chromosome numbers are $n=4$ in both *Crepis aspera* (Marchal 1920; Mann 1925) and *C. bursifolia* (Mann 1925). While there is not much difference in size between the *aspera* chromosomes, Mann found one chromosome in *bursifolia* considerably shorter than the others. She gives the comparative lengths as follows (determined on chromosomes in root-tip cells):

C. aspera	23.9	21.5	19.7	17.5
C. bursifolia	24.3	22.0	19.5	12.7

Except for the shortest one, the chromosomes of the two species apparently correspond in length.

During the stages of meiotic division the small chromosome pair of *C. bursifolia* can be recognized (pl. 58, figs. 12–15; pl. 59, figs.

16–18). A corresponding difference between chromosomes cannot be seen during meiosis of *C. aspera* (pl. 58, figs. 3–11). The pairing between the homologous chromosomes seems to be perfect in these two pure species, no irregularities being observed. As for the study of development of the gemini and structure of the chromosomes, certain species of *Crepis* are very favorable, the chromosomes being fairly large, of characteristic shape, and their numbers few. By using the described combination of Carnoy's and Navashin's fixatives and staining with iodine-gentian violet-orange, a very good and clear picture was obtained.

In early prophase single and rather thin threads of chromatin are seen in the nucleus (pl. 58, fig. 1). It is not a continuous spireme, as free ends are found, but even with the small number of 8 chromosomes in the cell it is difficult to determine the number of free ends, especially since the sections were differentiated considerably in order to show the structure of the chromosomes. The thread appears thickened at small intervals thus giving the appearance of a string of beads. Apparently these thickenings are real morphological units of the chromosome, namely, the chromomeres. In the stage shown in figure 1 the thread and the chromomeres probably are double, united in parallel pairs. This seems evident partly from the fact that the thread and the chromomeres are thicker here than in the early diplophase shown in plate 58, figure 2*b*, and partly because the thread in a stage a little later than figure 1 opens up and shows double in a few places (pl. 58, fig. 2*a*). The stage depicted in figure 1, therefore, is presumably to be regarded as a *zygophase* with the chromomeres from the parallel homologous chromosomes united two and two. Some places give the impression that the chromomeres are joined end to end by double threads.

In the *diplophase* four double units are seen in each of the two species (pl. 58, figs. 2–9, 12–13). To begin with, the partners of the gemini are somewhat irregularly twisted around each other several, up to 5–6, times (pl. 58, figs. 2–3). That each of these units represents a homologous *pair* of chromosomes is evident from the number, which is four. Thus the two constituent members of a given unit cannot be explained as the split halves of one chromosome but must necessarily be one of the partners of a conjugated *pair* of chromosomes. Parasyndesis is unquestionable in this case.

A little later the chromatin thread of the chromosome coils up into a more or less regular spiral, as one of the chromosome pairs in

plate 58, figure 4 clearly shows at one end; while the less clear parts of the chromosomes in this and in several other pollen mother cells appeared like a string of beads, the beads being much larger than in the earlier stages. Also the beads here apparently were not chromomeres but short spiral coils. Real spirals with tight windings, giving the impression of bars on a ribbon, might also sometimes mislead the observer.

As the diplophase proceeds, the paired chromosomes gradually uncoil and the chromosomes become shorter and thicker. This is probably due to the fact that the chromatic spiral of the chromosome, the *chromonema* as Vejdovsky, Kaufmann, and others name it, becomes larger in diameter. The four pairs of chromosomes in plate 58, figure 4 have not more than about two twists each.

In a little later phase, the typical *diaphase* (or diakinesis) each of the members contains two chromonemata or chromatids as shown in plate 58, figures 5, 8, and 13. The splitting for the homotypic division has here become visible, but the process of splitting may have taken place in an earlier phase. In several cases the spiral structure is very clear and regular, in other cases (perhaps a little later) the spirals are not so regular and tight (fig. 12). Although here the two chromonemata within one chromosome are a little twisted, we might with poorer staining get the impression of a *longitudinal split*, which is shown in so many pictures of chromosome division. This might also, in other cases, be due to the fact that the two spiral chromatids within the chromosome really have become separated from each other (Kuwada, 1926, 1927). The two zigzag spirals crossing one another within one chromosome also might sometimes give the appearance of alveolized chromosomes or of a reticulum.

As the chromosomes uncoil during the latter part of the diaphase there is often seen a connection between the two members of a chromosome pair. In several cases it can be seen that one of the spiral chromatids extends from one chromosome to its homologue while one of the chromatids from the latter chromosome extends to the former, the two connecting chromatids sometimes forming a cross where they change chromosomes (pl. 58, fig. 7). This phenomenon is very similar to the chiasmas which Janssens (1909, 1924) saw in the nuclei of insects and apparently is what Belling (1928*a*, p. 284) calls an X chiasma; but in *Crepis* the outline of the chromosomes is much more distinct than in Janssens' pictures, the nature of the connections, therefore, much less questionable.

The chiasmas must have been formed earlier but are not seen clearly before the two homologues uncoil. They might have been formed, as Belling suggests (1928*a*, pp. 288–290), by coincident breaks in two of the chromatids of the homologues and subsequent attachment of the wrong ends of the broken chromatids as text figure 1, *a*, *b*, shows. When the homologous (double) chromosomes are separated in heterotype meta-anaphase the rearranged chromonemata probably are pulled out of the chromosomes to which they originally belonged, as figure 1, *c*, *d*, indicates. In these figures no attention has been paid to the complication caused by the spiral twisting of the chromatids around one another or to the chromomeres.

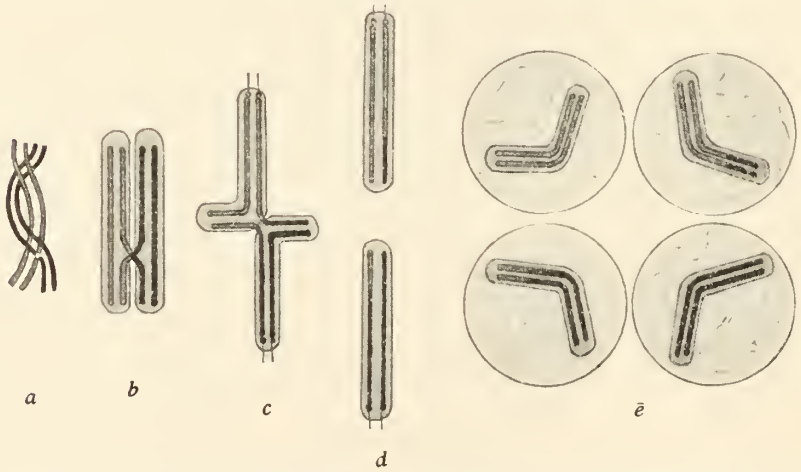

Fig. 1. Diagram illustrating the way crossing over might take place between two homologous chromosomes in the four-strand stage and chiasmas might be formed in accordance with the observations on *Crepis*.

a. The four strands twisted around each other in diplophase. A coincident break is shown in two chromatids from different homologues; the "matrix," in which the chromatids later on appear embodied, does not show in this stage, therefore the connection is more close.

b. Diaphase. The outline of the chromosomes shows up. An X-chiasma has been formed by wrong attachment of the two chromatids at the breaking-place.

c. Heterotypic metaphase; one of the two partners has turned around, a cross has been formed by pulling, but the chromosomes still have retained their individuality.

d. Heterotypic anaphase; the two chromatids with exchanged sections have been pulled out from their respective chromosomes and are enclosed within the matrix of the anaphasic chromosomes.

e. Tetrad stage; each of the four different chromatids has split longitudinally.

As will be understood, in all gemini in which chiasmas occur the X-crossed chromatids run from one chromosome to another. Which two of the four chromatids will go to either pole during anaphase will depend upon which two are together in the ends of the chromosomes

which have the fiber attachment. The disjunction of the spirally coiled and intercrossed chromatids must be a little difficult and in different organisms many anaphases show that the homologous chromosomes adhere very tightly to each other, sometimes showing a long connection between even rather widely separated homologues. It would be expected that exchanged parts of chromatids during this pulling would be straightened out and uncoiled to some extent.

The four gemini, in the diaphase of the two *Crepis* species mentioned, assume different shapes according to whether there is any connection between the two homologues or not, and according to where an eventual chiasma has taken place. So sometimes they form V's, sometimes X's, sometimes K's, and sometimes they are just parallel (pl. 58, figs. 5–9, 12–13). Figures 7 and 12 show gemini with presumably two connections (nodes, in Belling's terminology). Figure 8 shows a pair with a very conspicuous chiasma.

The shape of the bivalents during disjunction in the heterotypic meta-anaphase apparently is determined by their mode of connection during the late prophase. Plate 58, figures 10–11, shows this stage in *C. aspera;* figures 14 and 15, the corresponding ones in *C. bursifolia*. Only the small chromosome pair of *bursifolia* is rod-shaped and always very conspicuously different from the other pairs. Plate 59, figures 16–18, shows heterotypic anaphase and homotypic metaphase and anaphase of *C. bursifolia* in order to illustrate that the short chromosome of this species can be distinguished from the other three during all phases of division. During the phases following the diaphase the chromosomes stained so dark that it was impossible to see any structure in them. For the study of chromosome structure in these phases objects with less heavily staining chromosomes than those of *Crepis* should be used.

The facts observed regarding the spiral structure of the chromosomes in *Crepis* are a new link in the chain of observations made by many cytologists on different plants and animals, as for instance *Tradescantia* (Baranetzky, 1880; Kaufmann, 1926*a;* Kuwada and Sugimoto, 1926; Sakamura, 1927); *Ascaris* (Bonnevie, 1908; Vejdovsky, 1911–12), *Podophyllum* (Kaufmann, 1926*b*), *Fritillaria* (Newton, 1927), and *Vicia* (Kuwada, 1926, 1927). Also the zigzag filaments in chromosomes of *Paris* and *Listera* described by Martens (1922, 1924) might be considered evidence for a spiral structure. The occurrence of such structure very naturally explains how the chromosomes in the course of a short time change in form from long,

thin threads to short, thick rods. It also makes it more probable that the chiasmas described by several authors and hitherto mainly observed in animals may be the cytological evidence of crossing-over phenomena met with in genetical investigations. The chiasmas are observed in rather late prophases where the chromosomes are comparatively short and thick, and the fairly stable percentage of crossing over between certain genes would hardly be explained through exchange between elements so thick as the chromosomes are in this stage. But if the chiasmas are to be regarded only as evidence of an interchange or connection that has been accomplished before the chromosomes have been shortened by coiling up of the chromonemata, then it is more intelligible. Actual crossing over will then take place at an early stage but after the chromonema has been doubled to chromatids within the chromosome. The chiasmas will remain as an evidence of a crossing over that already has taken place, and the extraction of the crossed-over chromatids, finally, will not take place before the heterotypic anaphase.

Chiasmas in plants similar to those just described in *Crepis* have previously been shown to exist in *Uvularia* (Belling, 1926), *Tulipa* (Newton, 1927), and *Hyacinthus* (Belling, 1927). Newton dared not insist upon any interchange of parts of the chromatids, as the opening up of the four-strand group of chromatids in two different planes might explain the peculiar appearance. But in *Crepis* it hardly seems possible to explain the appearance of some gemini, as for instance the cross in plate 58, figure 8, in any other way than by admitting a segmental interchange between chromatids of homologous chromosomes. Here the outline of the chromosomes is seen clearly and there is no question as to which chromosome the different parts of the four chromatids belong. Thus far only one case of genetical crossing over has been observed in *Crepis* (Collins, 1924, p. 268). On the other hand *Crepis* is a favorable genus for a study of the chromosomal mechanism, and the occurrence of a haploid *C. capillaris* with only three chromosomes, and all three morphologically different, opens up a new field for a study of the early phases of meiosis, as Hollingshead (1928) has pointed out in her paper about the discovery of this haploid.

Chodat (1925) described in *Allium* a development of the heterotypic bivalent chromosomes from which he very ingeniously drew the conclusion that interchange between homologous chromosomes must take place, although in this case at the end of the chromosomes.

Not much of the structure of the chromosomes has been shown, and it is hard to be convinced that these big chromosomes really have exchanged parts at their ends, although the possibility of such exchange cannot be denied.

MEIOSIS IN THE HYBRIDS

1. *Crepis aspera* × *C. bursifolia.*—This hybrid was more extensively studied than the other two, because living material was obtainable for new fixation in the combination of Carnoy's and Navashin's fixatives in addition to the Carnoy-fixed material procured by Hollingshead.

The affinity between the chromosomes of the two species is not strong. Although in some cases 4 pairs of chromosomes can be found during the heterotypic division, more often only 3, 2, 1, or no pairs at all are found, as will be seen from table 1. Plate 59, figures 19–22, shows that spiral chromatids are seen within the chromosomes of the hybrids just as they are seen within the chromosomes of the parents during diaphase. As shown by figures 19–20, chiasmas can also be seen in the hybrid, indicating that crossing over might be found if offspring should be procured. Attention has been paid to the fact that an unequal pair ought to be found in all pollen mother cells with 4 pairs. This unequal pair, consisting of a small *bursifolia* and a larger *aspera* chromosome, can be traced all through. According to unpublished investigations of Hollingshead the small *bursifolia* chromosome is the one with a satellite. M. Navashin (1925, p. 107) observed that the satellites were connected with the nucleolus. In accordance therewith the unequal pair in *C. aspera* × *bursifolia*, when present as a pair, is found near the nucleolus (pl. 59, figs. 19–22). Whether the partner of the small *bursifolia* chromosome is the satellited *D*-chromosome from *C. aspera* (M. Navashin, 1927, fig. 4*c*) could not be settled, as the satellites do not show in the diaphase, but very probably it is that one because the pair is located at the nucleolus.

When an unequal pair is present, its partners are usually located end to end (pl. 59, figs. 19, 20, 22). This should indicate that no crossing over had taken place. Still, in a few cases there might be a possibility for a chiasma; thus in figure 21 the unequal pair seems to be parasyndetic at one end. A question which would be interesting to decide, is whether these two chromosomes unite at the satellited end or not. The chromosomes might be homologous with respect to this end.

TABLE 1

TYPES OF MEIOSIS I IN THREE *Crepis* HYBRIDS AS MANIFESTED BY THE OBSERVED POLLEN MOTHER CELLS

Hybrids	Number of bivalents and univalents					Total Number p. m. c.	Number of irregular p. m. c.	Per cent of irregular metaphase I	Number of chromosomes detached during meiosis I				Per cent p. m. c. with detached chromosomes
	^{4}II	^{3}II+I^{2}	^{2}II+^{4}I	^{7}II+^{6}I	^{8}I				0	1	2	3	
C. aspera×*bursifolia*	11	**24**	11	8	5	59	48	81.4	155	57	14	.....	31.4
C. taraxacifolia×*tectorum*	9	8	**11**	2	.....	30	21	70.0	4	9	9	1	(82.6)
C. aspera×*aculcata*	**22**	11	3	.....	.....	36	14	38.9	47	2	.....	.....	4.1

TABLE 2

IRREGULARITIES OBSERVED IN THE LATE TELOPHASE OF MEIOSIS II AND THE TETRAD PHASE IN THE THREE *Crepis* HYBRIDS

Figures indicate number of pollen mother cells.

Hybrids	Number of nuclei in late telophase of meiosis II									Other irregularities			Total of p. m. c. groups	Total irregular groups	Per cent of irregularities
	4	4+1 mic.	4+2 mic.	4+3 mic.	6	7	8	9	10	Pentads	Triads	Diads			
C. aspera×*bursifolia*	39	18	13	3	3	1	2	.....	1	2	2	1	85	46	54.1
C. taraxacifolia×*tectorum*	44	26	22	19	.....	1	3	1	.....	.....	3	1	120	76	63.3
C. aspera×*aculcata*	38	2	1	.....	.....	.....	.....	.....	.....	2	.....	.....	43	5	11.6

p. m. c.=pollen mother cell; *mic.*=micronucleus; *4+1 mic.* means 4 nuclei+1 micronucleus are observed in the pollen mother cell in question. The cases listed under 4 nuclei cover late telophases with 4 nuclei without any micronuclei and real tetrads without micronuclei and microcytes as well.

When only three pairs are present, the two univalent chromosomes usually are a short and a long one (pl. 59, fig. 24), but at least in one pollen mother cell with only three pairs, one of the pairs was unquestionably unequal (fig. 25). This shows that the affinity between the two members of the unequal pair is not much inferior to the affinity between some of the other chromosomes and it also shows that, when there is variation in number of bivalents, the chromosome pairs do not always follow the same consecutive order as regards pairing. The unequal pair shows much resemblance to an *XY* pair of sex chromosomes (pl. 59, fig. 29).

The small *bursifolia* chromosome can be recognized through heterotypic anaphase (pl. 59, fig. 30), homotypic metaphase (pl. 60, fig. 33), and anaphase (pl. 60, fig. 34). In figure 31, showing a heterotypic anaphase, it apparently is hesitating between the two poles and has split longitudinally. Unfortunately the hybrid is almost completely sterile. It sets only a few seeds by open pollination. Cytologically it would offer some advantages for a combined cytogenetic study, as the small *bursifolia* chromosomes can be followed through almost all phases of meiosis.

The most common case of pairing between the chromosomes in heterotypic metaphase is 3 bivalents + 2 univalents (table 1). Even if several univalents are present, the distribution of the chromosomes to the two poles in many cases will be 4 + 4 (table 3). This gives 4 chromosomes in most of the homotypic daughter nuclei (table 4).

TABLE 3

DISTRIBUTION OF THE CHROMOSOMES AS COUNTED FROM HETEROTYPIC ANAPHASES AND HOMOTYPIC METAPHASES IN *Crepis aspera* × *bursifolia* F_1
(3/1/4 means 3 and 4 to the two poles and one detached.)

Distribution of chromosomes	4/4	3/5	2/6	3/1/4	2/1/5	3/2/3	Total
Number of pollen mother cells	12	2	1	2	1	1	19

TABLE 4

NUMBER OF CHROMOSOMES IN HOMOTYPIC DAUGHTER NUCLEI OF *Crepis aspera* × *bursifolia* F_1

Number of chromosomes	6	5	4	3	2	Total
Number of nuclei	1	3	26	6	2	38

These counts are all from fixed and sectioned material. The deviation from the 4/4 distribution in several cases is caused by a detachment of one or two chromosomes (pl. 60, figs. 31, 32).

But the homotypic division and the following "tetrad" formation cause many irregularities as plate 60, figures 35–41, shows. Table 2 tells something about the nature of these irregularities. Usually the tetrad consists of 4 cells, but these may contain more than one nucleus. In such cases the extra nucleus or the extra nuclei are very small, apparently consisting of only one chromosome (pl. 60, figs. 37, 39). Sometimes these 4 cells are of very unequal size as figure 40 shows, the tetrad containing 4 cells but 8 nuclei. A pentad is shown in figure 38 and a triad with one large and 2 smaller nuclei in figure 41. Two pollen mother cells with a tendency to form diads are shown in figures 35 and 36. They have numerous nuclei. The "tetrad" stage as a whole gives a very irregular impression. It seems as if the walls circumscribing the cells that later become pollen cells are formed regardless of the nuclei, including 1, 2, 3, or perhaps 4 nuclei as the chance may be. In this way triads and diads may be formed. Probably most of the micronuclei degenerate.

There is an increase in amount of irregularity from the end of meiosis I, giving 31.4 per cent of pollen mother cells that have one or more chromosomes detached, to the end of meiosis II where 54.1 per cent of irregularities of different kinds are found (tables 1 and 2). To this percentage must be added the cases in which the irregularity does not manifest itself in the form of extra nuclei or in other ways but consists only in an unequal distribution of the chromosomes to four daughter nuclei as for instance $5 + 5 + 3 + 3$ instead of $4 + 4 + 4 + 4$.

It is hard to account for sterility in such a case as this. Even if irregularities are very common, still about 46 per cent of the pollen mother cells consist of only 4 nuclei, and a fairly large percentage of these ought to have 4 chromosomes. And also some of the irregular pollen mother cells ought to give gametes with 4 chromosomes. If only gametes containing the same sets of chromosomes as the pure parental species are viable, then the sterility can be accounted for satisfactorily. Out of 16 gametes with 4 chromosomes one gamete ought to give a pure set of *aspera* and one a pure set of *bursifolia* chromosomes. Each floret has only one ovule and if the irregularities in the formation of the eggs are similar to those for formation of pollen there should be expected one ovule with a pure set of *aspera*

and one with a pure set of *bursifolia* chromosomes among 40 to 50 florets. If about the same amount of pollen—good and bad together—were available for each ovule, or if F_1 were backcrossed with pollen of one of the parent species, we should expect at least 4 or 5 per cent of the florets to set seed giving either pure *aspera*, pure *bursifolia*, or the F_1 hybrid type again. But among the gametes calculated to contain either *aspera* or *bursifolia* chromosomes alone, some might contain chromosomes that have been changed by crossing over, so that the balance has been disturbed. The *Crepis* chromosome groups do not seem to withstand even a small change in balance as well as do, for instance, the chromosome groups in certain *Viola* hybrids (J. Clausen, 1926). All these things ought to account for the small degree of fertility. Haney estimated the fertility to be about 3–4 per cent by open pollination. No seeds have been collected.

The type of meiosis described for this *Crepis* hybrid is dissimilar to the type described by M. Navashin (1927) for the hybrid *C. capillaris* × *aspera*. In this last-mentioned hybrid lengthwise splitting apparently is a common phenomenon.

2. *Crepis taraxacifolia* × *C. tectorum*.—Meiosis in this hybrid is similar in general to that in the hybrid just described. Of *C. taraxacifolia* × *tectorum* only iron-acetocarmine smears have been used and only from material fixed in Carnoy's fluid by Hollingshead. An attempt to make permanent, sectioned slides from this fixation was unsuccessful. No differentiation could be obtained either with iodine-gentian violet or with Heidenhain's iron-haematoxylin. The iron-acetocarmine could be used but did not give good differentiation.

From table 1, page 412, it will be seen that the most common situation met with in heterotypic metaphase was 2 bivalents and 4 univalents, while 70 per cent of heterotypic metaphases are irregular. Detachment of chromosomes during meiosis I is common and there are 63 per cent of irregularities in the tetrad phase. Plate 61, figures 42–45, gives different heterotypic metaphases with from 4 to 2 pairs of chromosomes. No visible difference in size is here discernible among the chromosomes. Plate 61, figure 46, shows a regular, and figure 47, an irregular homotypic anaphase, while figure 48 is a schematic figure drawn to show how the chromosomes in figure 47 would be distributed among the nuclei a little later. Figures 49 to 54 show how the tetrad phase looks in this hybrid. Figure 49 is a nonad. As only 16 chromosomes will be present in the nine nuclei altogether, it is not hard to figure out how many chromosomes will

be in each of these nine nuclei; five of them ought to have 1 chromosome each, one would have 2 chromosomes and three ought to have 3 chromosomes each. Figure 52 shows an unquestionable diad and figures 53 and 54, triads, figure 54 having 4 nuclei but only 3 cells; figure 51 shows a tetrad with cells unequal in size. The amount of fertility is about the same in this hybrid as in the preceding one, according to Haney. Table 5 gives an analysis of 13 pollen mother cells in homotypic anaphase by direct count of chromosome number. Actually a regular distribution of the chromosomes seems to be fairly common. Table 6 shows that 66.6 per cent of the nuclei observed in these 13 pollen mother cells have 4 chromosomes. One fact must here be borne in mind, namely, that pollen mother cells with a regular distribution of the chromosomes will show up best and are more likely to be countable than those with fairly irregular distribution of chromosomes. If no crossing over took place and if 66.6 per cent of the gametes have 4 chromosomes, then 8.3 per cent of the gametes ought to have either a pure *taraxacifolia* or a pure *tectorum* group of chromosomes. The plants exhibited exceedingly low fertility but this may have been partly caused by the unfavorable season at which they reached maturity.

In this hybrid no chromosomes can be distinguished during the meiotic divisions as belonging to either of the parental species. They are all of practically the same length.

3. *Crepis aspera* × *aculeata*.—This hybrid is conspicuously different from the first two as regards the number of irregularities during meiosis, being much more regular. As in *taraxacifolia* × *tectorum*, the chromosomes of the two parental species cannot be distinguished during meiosis. Four bivalent chromosomes are most commonly observed in the heterotypic metaphase; only 38.9 per cent of the pollen mother cells show irregularities during this phase; and only 4.1 per cent of the pollen mother cells have chromosomes detached through meiosis 1 (table 1, page 412). From table 2 it will be seen that only 11.6 per cent of the pollen mother cells in the tetrad phase have irregularities. The hybrid is fairly fertile; it is estimated that 35 to 40 per cent of the florets set seed.

Plate 61, figures 55–58, shows some of the division figures, mainly of the relatively few irregular ones. Figure 57 shows a heterotypic anaphase with the distribution of 4 chromosomes to one pole, 3 to the other, and 1 chromosome between the plates, splitting. Figure 58 is a tetrad with a micronucleus in each of two of the cells.

Only iron-acetocarmine smears from Carnoy-fixed material have been used in the investigation of this hybrid, also, because no differentiation was obtainable in the imbedded and sectioned material.

TABLE 5

DISTRIBUTION OF CHROMOSOMES IN HOMOTYPIC ANAPHASE OF *C. taraxacifolia* × *tectorum* F_1

Number of chromosomes in the four nuclear plates						Number of p. m. c.
A	Between the plates	A_1	*B*	Between the plates	B_1	
4	.....	4	4	.....	4	5
4	.....	4	4	.....	×	2
3	2	3	3	2	3	1
3	2	3	4	1	3	1
3	2	3	3+½	1	3+½	1
3	2	3	4	.....	4	1
5	.....	5	3	.....	3	1
4	1	3	×	1	×	1
					Total	13

×, means that the nuclear plate in question could not be counted.
A and A_1, *B* and B_1 are corresponding nuclear plates.

TABLE 6

NUMBER OF CHROMOSOMES IN 48 HOMOTYPIC ANAPHASE NUCLEI AS CALCULATED FROM TABLE 5 (DIRECT OBSERVATION)

	Actual counting			Total
Number of chromosomes	3	4	5	.....
Number of nuclei.....	14	32	2	48
Per cent	29.2	66.6	4.2	100

The type of conjugation described in the *Crepis* hybrids, with a fairly wide range of variation in the amount of pairing between the chromosomes, has been observed by the junior author in a number of *Viola* hybrids. Apparently in these cases *some* affinity must exist between all the partly homologous chromosomes as they sometimes all pair. Still the affinity must be somewhat weaker as the chromosomes often fail to conjugate. These cases of partial affinity between the chromosomes are intermediate between two extremes. On one hand we have hybrids in which the affinity between the parental chromosomes is so slight that they will *never* conjugate, as, for instance, in the hybrids *Nicotiana Bigelovii* × *suaveolens* and *Bigelovii* × *glutinosa* (Goodspeed and Clausen, 1927) and *Raphanus* × *Brassica* (Kar-

pechenko, 1924). The other extreme is represented by hybrids in which the affinity between the parental chromosomes is so strong that they *always* pair. This condition can be illustrated by the hybrids *Geum rivale* × *urbanum*, both with $n = 21$ chromosomes, and *Tragopogon pratensis* × *porrifolius*, both $n = 6$ (Winge, 1926, 1928), and by varietal hybrids. Very similar to this is the case where the two parents have different chromosome numbers but *all* chromosomes from the parent that has the lowest chromosome number, pair with their homologues from the other parents, as in *Nicotiana paniculata* × *rustica* (Goodspeed, Clausen and Chipman, 1926); and in all clear cases following the *Drosera* scheme. Between these two extremes are the cases described in the three *Crepis* hybrids. Apparently a large gradation exists between these extremes as manifested by the different percentages of failures to pair in different hybrids. Although in the *Crepis* case the degree of sterility to some extent seems to follow the degree of cytological irregularities and the degree of failure to conjugate, it does not always hold true, as shown by the *Tragopogon* hybrid which was almost sterile notwithstanding complete pairing; and similarly in *Lamium* hybrids described by C. A. Jörgensen (1927). The sterility might be caused mainly by non-balanced interaction of genes and perhaps has not much connection with the degree of mutual affinity between chromosomes. Only so far as mutual affinity between homologous chromosomes is *one* of the factors responsible for keeping up an *already established* balance between the genes constituting a certain genotype, can it be said that affinity between chromosomes has any bearing upon the question of sterility.

The present paper in all essentials was completed May 15, 1928, but was delayed five months by overwhelming experimental work. In the meantime two papers bearing on similar phenomena were published, namely, Maeda's paper on *Lathyrus* (1928) and Belling's paper on *Lilium* (1928*b*). Maeda also has applied the combination of Carnoy's and Navashin's fixatives for bringing out very conspicuously the spiral structure of the chromosomes. Belling's description of the chiasmas in *Lilium* (pp. 467–468) corresponds with the condition in *Crepis*, but the figures, as well as the earlier figures of *Uvularia* and *Hyacinthus*, apparently do not show such fine details as *Crepis* shows. Whether this is due to a profound difference in structure of the chromosomes or to the squeezing applied in Belling's investigations cannot be told.

TAXONOMIC RELATIONS OF THE FIVE SPECIES

According to a tentative taxonomic grouping (Babcock and Lesley, 1926, slightly modified) the five species discussed here would be classified as follows:

SUBGENUS A. Achenes beaked

Sec. III. **Barkhausia**
Crepis bursifolia
Crepis taraxacifolia

Sec. IV. **Nemauchenes**
Crepis aspera
Crepis aculeata

SUBGENUS B. Achenes not beaked

Sec. VI. **Eucrepis**
Crepis tectorum

The above sections are distinguished primarily by the form of the achenes. In *Barkhausia*, as tentatively used here, the achenes are all similar and definitely beaked. In *Nemauchenes* they are of two shapes, the marginal achenes being unbeaked and the inner ones beaked. In *Eucrepis* the achenes are all unbeaked (or in a few species very shortly beaked). The achenes of all but one of these five species have been illustrated (Babcock and Lesley, 1926, fig. 3, *g*, *h*, *h'*; fig. 5, *r*). In *Crepis aculeata* the achenes resemble those of *C. aspera* but are larger, while the marginal ones are less prominently angled and the inner ones proportionately shorter beaked.

Considering the meiotic behavior and fertility of the hybrids herein discussed with reference to classification of the species involved, it is clearly shown in tables 1 and 2 that the *Crepis aspera* × *aculeata* hybrid displays the lowest amount of chromosome irregularities in meiosis I with a large amount of regular pairing and very few pollen mother cells showing detached chromosomes. The percentage of irregularities in meiosis II is also low. In the *C. taraxacifolia* × *tectorum* F_1, on the other hand, less than one-third of the pollen mother cells examined show regular pairing and a very large percentage have detached chromosomes. The *C. aspera* × *bursifolia* F_1 showed even less regularity in pairing in its pollen mother cells, but the number of detached chromosomes observed was not so high as in

the *taraxacifolia* × *tectorum* hybrid, while the percentage of irregularities in meiosis II was nearly as large. In other words, the last two hybrids, one involving different subgenera and the other, different sections of the same subgenus, display much greater irregularity during meiosis in their pollen mother cells than does the hybrid between two species of the same section.

Data on the proportion of apparently good pollen grains formed are in close agreement with the foregoing. Pollen counts of 500 to 1000 grains per plant were made in most cases. The pollen was stained with acetocarmine and showed great variation in size and staining capacity of the grains. Only those grains of average or large size and deeply stained were counted as "good." In the case of *C. aspera* × *aculeata* three plants gave 36, 40, and 48 per cent respectively of good pollen. Of the *taraxacifolia* × *tectorum* hybrids three plants had from 1 to 2 per cent of good grains. Only one plant of *aspera* × *bursifolia* was examined and it gave less than 1 per cent of good pollen. Fortunately, however, pollen counts were made on three plants of *bursifolia* × *aculeata* and two plants of *taraxacifolia* × *aspera* and all these gave only 1 per cent or less of good pollen.

The proportion of viable egg cells produced in *Crepis* hybrids is usually larger than the proportion of viable pollen formed. The only data bearing on this in the three hybrids discussed above are Haney's observations on the amount of open-pollinated seed produced, and under conditions at Berkeley this is rather variable. The observations made, however, indicate general agreement between amount of open-pollinated seed produced and proportion of good pollen grains present.

The data on meiosis, pollen formation, and fertility may be summarized as follows:

Subgenus	Section	Hybrids	Meiotic irregularities observed	Percentage of "good" pollen formed	Percentage of seeds set under open-pollination
A	IV	*aspera* × *aculeata*	Rather few	35–50	30–40
A	III	*taraxacifolia* × *tectorum*	Very numerous	1–2	Few or none
B	VI				
A	IV	*aspera* × *bursifolia*	Very numerous	1±	3–4
A	III				

These results are in fairly good agreement with the taxonomic relationship as determined by comparative morphology. It will be noted, however, that meiotic irregularities are just about as high and fertility nearly as low in *aspera* × *bursifolia* (both in Subgenus *A*) as in *taraxacifolia* × *tectorum* (Subgenus *A* × *B*). This must indicate profound physiological diversity between *Barkhausia* and *Nemauchenes*. It provides additional reason for maintaining these species of *Nemauchenes* as a separate group from their close relatives in *Barkhausia*. It also shows, however, that when the relationship between two species is below a certain threshold value, the meiotic irregularities and degree of sterility exhibited by hybrids between them are of little value in determining the degree of relationship between the species. These criteria, in other words, are useful only within certain limits in the study of taxonomy, and should be considered only in relation to other criteria such as number and morphology of the chromosomes, and the distribution, ecology, and comparative morphology of the plants themselves.

SUMMARY

1. This paper deals with the two pure species, *Crepis aspera* L., and *C. bursifolia* L., together with the following three hybrids: *Crepis aspera* × *C. bursifolia*, *C. taraxacifolia* Thuill. × *C. tectorum* L., and *C. aspera* × *C. aculeata* (DC.) Boiss.

2. All five species have $n = 4$ chromosomes. After a discussion of methods of fixation and staining, meiosis in the two species, *C. aspera* and *C. bursifolia*, is described. In *C. bursifolia* one chromosome pair, which is shorter than the other three pairs, can be recognized through all phases. The chromonema thread in the early zygophase shows chromomeres, and parasyndesis has been found. In the diplophase the chromonema coils up into a spiral filament which in the diaphase or diakinesis appears to be doubled by splitting. In this phase chiasmas between the partners of the bivalent chromosomes are often seen. These chiasmas may be regarded as the result of interchange accomplished at an earlier stage, before the chromosomes have become shortened by the spiral coiling of the chromonemata. Extraction of the crossed-over chromatids would occur in the heterotypic anaphase.

3. In the hybrid, *C. aspera* × *bursifolia*, the structure of the chromosomes can be recognized as similar to that of the parent species and chiasmas can be found between the partners of the bivalent chromosomes. In pollen mother cells where four pairs of chromosomes are present, a pair consisting of two unequal partners is seen and the short *bursifolia* chromosome can be followed through and recognized in all phases.

4. Of the three hybrids, *C. taraxacifolia* × *tectorum* and *C. aspera* × *bursifolia* show comparatively many irregularities during meiosis, while *C. aspera* × *aculeata* is fairly regular (table 1 and 2, p. 412).

5. The data on meiotic irregularities in these hybrids are in close agreement with the taxonomic relations between the parental species; also the data on percentage of "good" pollen formed in the hybrids, and their fertility, agree well, in the main, with the irregularities observed in meiosis as well as with the taxonomic relations, with the exception that, if their relationship is below a certain threshold value, these criteria cannot be used for determining how remote two species are from each other.

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EXPLANATION OF PLATES

For slides of sectioned material Leitz' 2 mm.-apochromatic objective, aperture 1.4, together with binocular eye-piece × 15 has been applied; for iron-acetocarmine smears, Leitz' 3 mm.-apochromatic objective, together with the same eye-piece. Parallel rays from a small circular source of light of great intensity were sent through Leitz' achromatic condenser aperture 1.4 for illumination.

The magnification of sectioned material is about × 2100. In the preparations treated with acetocarmine the swelling of the chromosomes caused by the acetic acid just compensates the minor magnification of the 3 mm.-objective as compared with the 2 mm.-objective, so that all pollen mother cells reproduced appear of practically the same average size.

In the tetrads and polyads stress has been laid mainly upon number, size, and shape of nuclei and cells, and the structure shown in the nuclei of these must be regarded only as a kind of signature.

PLATE 58

Crepis aspera.

Fixation: Carnoy-Navashin. Sectioned material. Stained in gentian violet-iodine-orange G.

Fig. 1. Zygophase; the thick chromomeres probably have been formed by parallel union of pairs of chromomeres.

Fig. 2. *a*. The chromatic thread opens up, showing its doubleness; *b*, one of the four bivalents in diplophase, a little later. Seven twists of its partners around each other were counted; note the chromomeres.

Fig. 3. The four bivalents in one pollen mother cell in diplophase; the partners still much twisted around each other.

Fig. 4. Later diplophase; four bivalents are seen; the partners untwist but the chromatic thread coils up in a spiral.

Figs. 5–9. Diaphases (diakinesis). In all pollen mother cells 4 bivalents can be counted. Note the two spiral chromatids in each chromosome in this phase. Chiasmas are clearly seen in figures 5–8; in figure 6 one bivalent with a chiasma is drawn outside the nucleus in order to show the underlying bivalent; in figure 7 one bivalent probably has two chiasmas; in figure 8 there is one clear chiasma, the two partners forming a cross. Figure 9 shows different shapes of bivalents in one pollen mother cell.

Figs. 10–11. Two heterotypic metaphases; different shapes of bivalents.

Crepis bursifolia.

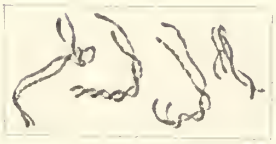
Sectioned material; fixed and stained as the preceding ones.

Figs. 12–13. Diaphases of two pollen mother cells; different shapes of bivalents (rings, crosses). Note the short pair and the spiral chromatids.

Figs. 14–15. Two heterotypic metaphases, each with 4 bivalents; in figure 14 three crosses and perhaps one rod; in figure 15 a double J, two rings and one rod. Note the short bivalent.

1

2a 2b



3

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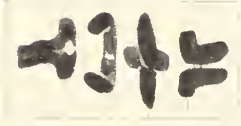

11

12

13

14

15

PLATE 59

Crepis bursifolia.

Fig. 16, heterotypic anaphase; fig. 17, homotypic metaphase; fig. 18, homotypic anaphase; note the short chromosome in all four groups of chromosomes.

Crepis aspera × *bursifolia*, F_1.

Sectioned material. Fixation: Figure 30, Carnoy; all the others Carnoy-Navashin and all stained in gentian violet-iodine-orange G.

Figs. 19–22. Selected diaphases in which four bivalents occur. Note the conjugation of the unequal pair and the chiasmas in figures 19–21; in figure 20 it has been necessary to draw one bivalent outside the nucleus.

Figs. 23–29. Heterotypic metaphases. Figure 23 has 4_{II}, figures 24–25 $3_{II} + 2_I$, figure 26 has $2_{II} + 4_I$; in figure 27 there is $1_{II} + 6_I$ and in figure 28, finally, all 8 chromosomes are unpaired (non-reduction). In figure 23 probably the left bivalent is the unequal one; in figure 24 the two unpaired chromosomes are the unequal ones, in figure 25 these are conjugated, resembling a pair of *XY* chromosomes, while two equal chromosomes here are unpaired. In figure 29 (not complete) an unequal pair very clearly shows.

Fig. 30. Heterotypic anaphase giving 4 long chromosomes in one of the daughter nuclei and 3 long and one short in the other.

16

17

18

19

20

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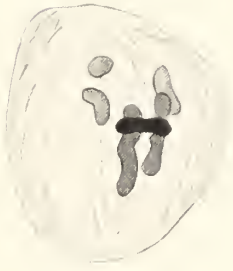

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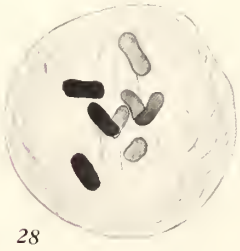

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PLATE 60

Crepis aspera × *bursifolia*, F_1.

Figures 31–34 from sectioned material, figure 31 from Carnoy fixation, the others from Carnoy-Navashin, all stained in gentian violet-iodine-orange G. Figures 35–41 are from iron-acetocarmine smears, previously fixed in Carnoy and preserved in 70 per cent alcohol.

Fig. 31. Heterotypic anaphase; one chromosome eliminated, the short chromosome splitting in the equatorial plane.

Figs. 32, 33. Homotypic anaphase. Figure 32 has five chromosomes at one pole, two at the opposite pole, and one (the small one?) splitting outside both daughter groups of chromosomes.

Fig. 34. Homotypic anaphase in one section; the left picture represents the upper cap of the pollen mother cell. The short chromosome is seen in this cap.

Figs. 35–41. Polyads representing the tetrad stage. Figure 35 apparently will form a diad with two and four nuclei respectively. Figure 36 probably represents a diad, but possibly it might belong to interphase (interkinesis); three micronuclei are seen. Figures 37 and 39 are tetrads with micronuclei. Figure 28 is a pentad, figure 40 a tetrad, but one of its cells is very small and one is very large with three micronuclei. Figure 41 is a true triad.

Crepis taraxacifolia × *tectorum*, F_1.

Smear preparations fixed previously in Carnoy and stained in iron-acetocarmine.

Figs. 42–45. Heterotypic metaphases. In figure 42, 4_{II}; in figure 43, $3_{II} + 2_I$. Figures 44 and 45 have $2_{II} + 4_I$ each, but in figure 44 the bivalents form a cross and a rod, respectively, while in figure 45 they form two crosses.

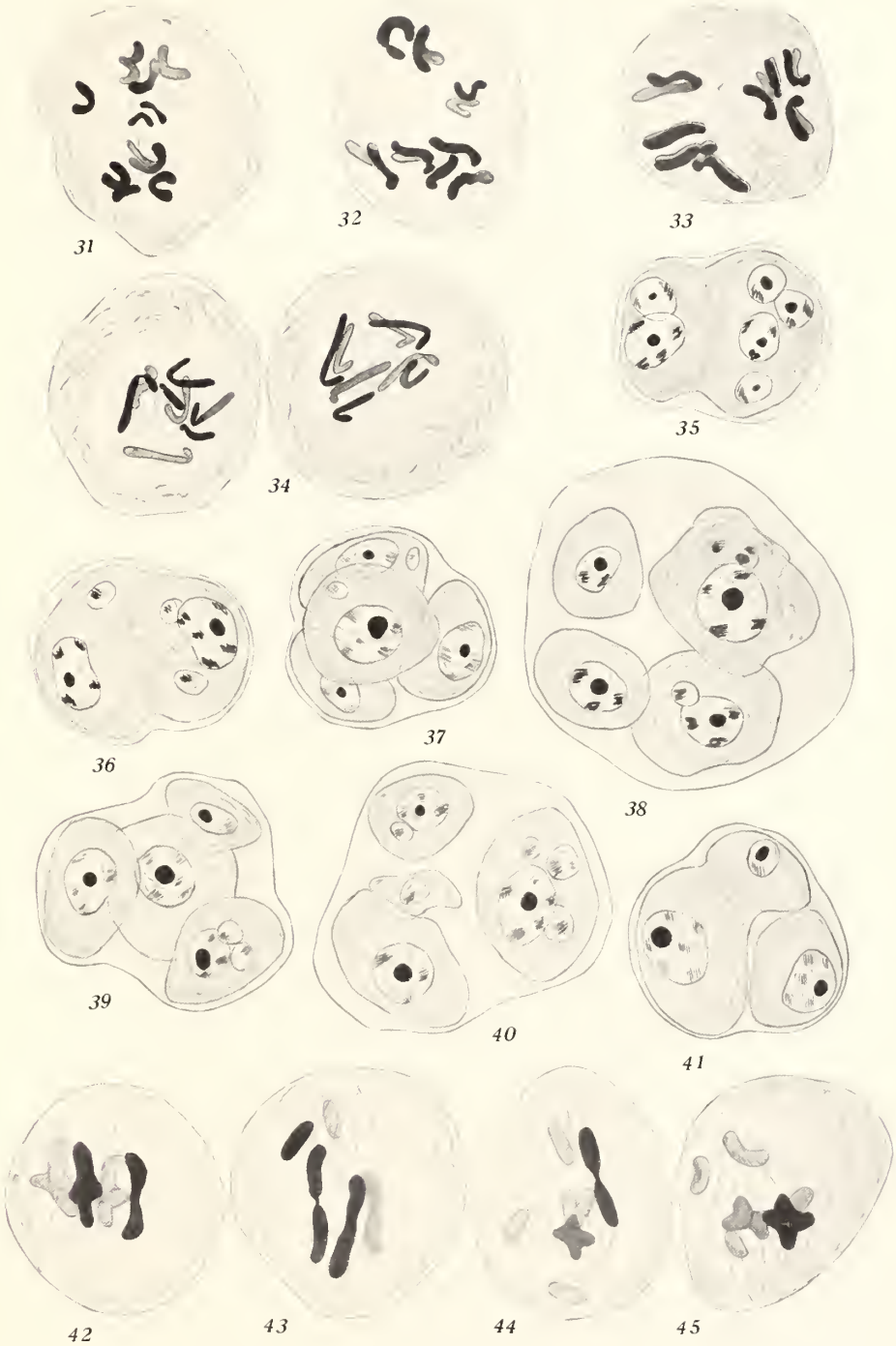
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PLATE 61

Crepis taraxacifolia × *tectorum*, F_1.

Fig. 46. Homotypic anaphase with four chromosomes in all four groups.

Fig. 47. Homotypic anaphase with 3/2/3 chromosomes in the upper of the two division figures and 4/1/3 in the lower one.

Fig. 48. Represents the tetrad that probably would result from the division shown in figure 47, the figures in the nuclei giving the number of chromosomes in the four nuclei and three micronuclei.

Fig. 49. A nonad of which three nuclei probably have three chromosomes each, one probably has two chromosomes, and five are micronuclei with probably only one chromosome each, giving the total of sixteen chromosomes in the nine nuclei.

Figs. 50–51. Tetrads with micronuclei; in figure 51 one of the cells is very small.

Fig. 52. A true diad.

Figs. 53 and 54. Triads; in figure 54 one of the cells has two nuclei.

Crepis aspera × *aculeata* F_1.

Iron-acetocarmine smears. The material previously fixed in Carnoy.

Figs. 55–56. Heterotypic metaphase; in figure 55 4_{II} (the most common case), in figure 56 $3_{II} + 2_{I}$.

Fig. 57. Heterotypic anaphase; one chromosome lagging and splitting.

Fig. 58. Tetrad; one of the few cases where micronuclei were found.

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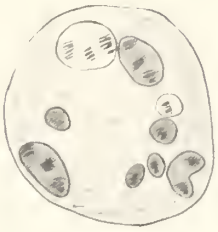

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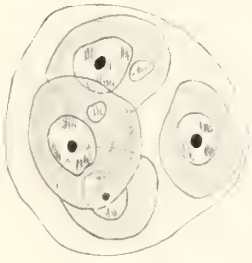

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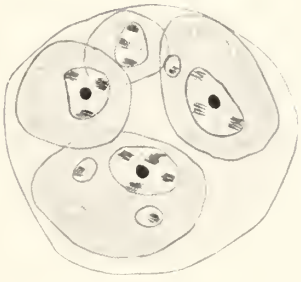

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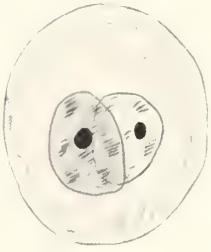

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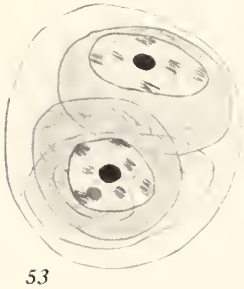

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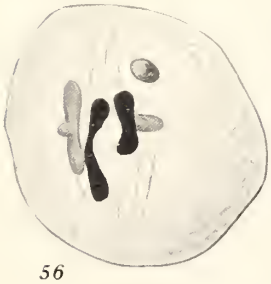

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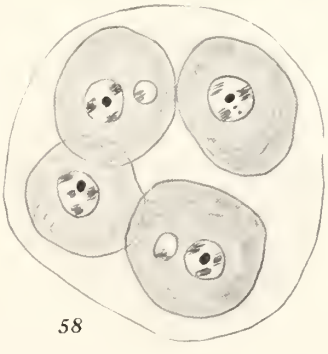

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